US ERA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

12-30

1. CHEMICAL: Dithane M-45

SN: 014504

2. TEST MATERIAL:

The test material used in this study was technical Dithane M-45, Lot # 76777, containing 82.4 percent active ingredient.

3. STUDY/ACTION TYPE: Acute shrimp LC50 study

4. STUDY IDENTIFICATION:

Ward, G.S. Acute Toxicity of Dithane M-45 Fungicide to Mysid Shrimp (Mysidopsis bahia) Under Static Conditions. 1988. Environmental Science and Engineering Inc. Project ID # 87369-0300-2130. Study sponsor: Rohm and Haas Company. Study location: Gainesville, FL. EPA Acc. No. 405868-01.

5. REVIEWED BY:

Robert W. Pilsucki, Microbiologist Ecological Effects Branch Hazard Evaluation Division

Signature:
Date: 4/16/88

6. APPROVED BY:

Raymond W. Matheny, Head, Section 1 Ecological Effects Branch Hazard Evaluation Division

Signature: (Jay W. Mather

Date:

JUN 1 7 1988

7. CONCLUSIONS:

Because the concentration of the test material was not measured after a substantial decrease in dissolved oxygen and subsequent aeration, the test is considered supplemental. The reported LC50 was 67 (95% C.L. = 58 and 79) ppb. This study does not fulfill the guideline requirement for an acute shrimp LC50 test.

8. RECOMMENDATION:

Additional information from the first test, if not aerated, may be used in conjunction with the results of this test in order to fulfill the guideline requirement.

9. BACKGROUND:

This study was submitted in response to the Mancozeb Registration Standard data call in.

10. DISCUSSION OF INDIVIDUAL STUDIES OR TESTS: NA

11. METHODS AND MATERIALS:

Species. Mysid shrimp (Mysidopsis bahia)

Age. 4 - 6 days

Source.

The shrimp were from Environmental Science and Engineering's stock cultures.

Test vessel.

Size/Volume: 1.6 L Carolina dishes containing 1 L of solution. The depth was approximately 4 cm.

Loading: Not determined

Test water.

Temperature: 19 - 23 OC

Water source and chemistry: The water used for the test was natural seawater collected at Marineland, FL, which was subsequently filtered through a 5 micron filter. The water was diluted to approximately 20 ppt for the test. See attached table for full characterization of the water.

Aeration: One hour at the 72-hour mark.

Solvent.

The solvent used was seawater.

Controls.

There was a nondosed control group, consisting of 20 individuals.

Number of shrimp per concentration. 20

Toxic signs

Shrimp were observed daily for mortality, toxic signs and abnormal behavior.

Statistical analysis

Mortality was analyzed using Stephan's computerized program.

12. REPORTED RESULTS:

Concentration (ppb)	Number Exposed	Number Dead	Percent Mortality
167	20	20	100
100	20	19	95
60	20	7	35
36	20	0	0
22	20	0	0
13	20	1	5
Control	20	0	0

The range-finding test, using concentrations between 30 and 3,000 ug a.i./L indicated that the LC50 was below 30 ug/L. Therefore, values between 13 and 167 ug. a.i./L were chosen for the definitive test.

In the first definitive test, unfiltered seawater was used, resulting in noticeable sedimentation. The test was repeated using filtered seawater.

The second definitive test showed no control mortality. At the highest two concentrations (100 and 167 ppb) most shrimp began showing signs of toxicity (lethargy and complete loss of equilibrium) at 24 hours. Mortality was 95% and 100% at 100 and 167 ppb respectively. The LC50 for the second test was reported as 67 (95% C.L. = 58 and 79) ppb. The author also reported the LC50 obtained in the first definitive test: 37 (95% C.L. = 32 and 43) ppb. There was one mortality at the 22 ppb which, the author stated was ignored for the no-observed effect concentration (NOEC) determination. The NOEC was determined to be 37 ppb.

The author reported that salinity of the water remained at 20 ppt during the test. The mean temperature during the test was 20.7 $^{\circ}$ C (s = 1.8 $^{\circ}$ C). Dissolved oxygen remained >70 percent during the first 48 hours but dropped to 28 percent at 72 hours

whereupon the test solutions were gently aerated for one hour, resulting in a DO of >86 percent. Dissolved oxygen at test's end was >46 percent. The pH ranged from 7,8 to 8.2 in all test aquaria.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The author made no formal conclusions. There was a quality assurance statement attached to the study.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF THE STUDY:

A. Test Procedure.

The procedure follows EPA's <u>Pesticide Assessment Guidelines</u>: <u>Subdivision E</u> except for the following:

- 1. the shrimp were acclimated for only 4 6 days instead of the recommended 10 days;
- 2, the temperature was outside the recommended range (21 23 $^{\rm O}{\rm C}$) during the first two days of the test; and
- 3 the test solutions were aerated during the study.

B. Statistical Analysis.

Validation of the statistical analysis produced an LC50 that agrees closely with that of the author's.

C. Results/Discussion.

The temperature deviation was slow and slight and, according to the author's belief, did not affect the outcome of the test. EEB agrees that the slight deviation in temperature probably did not overly stress the test animals.

The second deviation, the reduced acclimation period, probably did not affect the study, since there were no control moralities and the shrimp were observed to be healthy at the beginning of the test and control shrimp showed no obvious abnormalities during the study.

Aeration of the test vessels, even though only for an hour, may have confounded the results of the second definitive test, especially since the first definitive test showed a lower 96-hour LC50. Aeration, depending on the test material's volatility, can significantly reduce the actual concentration of the test material in solution. There was no measurement of the test material after aeration and no discussion of what may have caused

the severe reduction in dissolved oxygen in both treatment and control vessels.

The author noted that the first test was an acceptable test and the only reason for repeating it was sediment in the seawater. If this first test was un aerated, it is possible that the results from that test can be used, along with this test to fulfill the data requirement.

D. Adequacy of the Study.

- 1. Category: Supplemental
- 2. Rationale: Aeration may have altered the concentration of the test material in solution. The concentration of test material was not measured after aeration.
- 3. Repairability: Additional information from the first test, if not aerated, may be used in conjunction with the results of this test in order to fulfill the guideline requirement.
- 15. COMPLETION OF ONE-LINER One-liner completed 06-14-88.

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CONC.	NUMBER	NUMBER	PERCENT	BINOMIAL
	EXPOSED	DEAD	DEAD	PROB (PERCENT)
167	20	20	100	9.536742E-05
100	20	19	95	2.002716E-03
60	20	7	35	13.1588
36	20	0	0	9.536742E-05
22	20	0	0	9.536742E-05
13	. 20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 36 AND 100 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER-THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 67.08821

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN G LC50 95 PERCENT CONFIDENCE LIMITS

4 5.135008E-02 67.3667 57.85791 79.40593

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS G H GOODNESS OF FIT PROBABILITY
6 8.681122 43.25896 0
A PROBABILITY OF O MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 5.018481 95 PERCENT CONFIDENCE LIMITS =-9.767843 AND 19.8048

LC50 = 62.89018 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

Pages through are not included.
The material not included contains the following type of information:
Identity of product inert ingredients.
Identity of product impurities.
Description of the product manufacturing process.
Description of quality control procedures.
Identity of the source of product ingredients.
Sales or other commercial/financial information.
A draft product label.
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Information about a pending registration action.
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